The Auk 125(4):839–849, 2008

Printed in USA.

GENETIC DIVERGENCE AND DIFFERENTIATION WITHIN THE WESTERN SCRUB-JAY (*APHELOCOMA CALIFORNICA*)

KATHLEEN SEMPLE DELANEY,[1] SABA ZAFAR, AND ROBERT K. WAYNE

Department of Ecology and Evolutionary Biology, University of California, 621 S. Charles E. Young Drive, Los Angeles, California 90095, USA

ABSTRACT.—Western Scrub-Jays (*Aphelocoma californica*) range throughout the western United States and Mexico and exhibit substantial geographic variation in morphology and behavior. Western Scrub-Jays are a polytypic species complex with three distinct groups (*californica*, *woodhouseii*, and *sumichrasti*) associated with geography and distinguished by morphology, genetics, and behavior. Until recently, the Island Scrub-Jay (*A. insularis*) and the Florida Scrub-Jay (*A. coerulescens*) were considered subspecies within the Western Scrub-Jay complex. We analyzed mitochondrial DNA sequences (control region I) to reveal the phylogenetic relationships within Western Scrub-Jays and used maximum likelihood, a Bayesian approach, and a relaxed phylogenetic approach to construct phylogenies. In addition, we used several methods to estimate divergence time between clades. Our results reveal that the interior *woodhouseii* group, which contains the *sumichrasti* subclade, and the coastal *californica* group have had a long and independent evolutionary history. However, samples from a contact zone in western Nevada were genetically *woodhouseii* and morphologically *californica*. Island Scrub-Jays evolved from the coastal *californica*, which makes Western Scrub-Jays paraphyletic with respect to Island Scrub-Jays. To solve the problem of paraphyly and to more accurately define the diversity that exists within Western Scrub-Jays, we suggest splitting Western Scrub-Jays into two or three species, California Scrub-Jay (*A. californica*), Woodhouse's Scrub-Jay (*A. woodhouseii*), and, potentially, Sumichrast's Scrub-Jay (*A. sumichrasti*). *Received 30 May 2007, accepted 6 March 2008.*

Key words: *Aphelocoma californica*, genetic divergence, mtDNA sequences, paraphyly, phylogeography, Western Scrub-Jay.

Divergencia Genética y Diferenciación dentro de *Aphelocoma californica*

RESUMEN.—*Aphelocoma californica* se encuentra en todo el oeste de los Estados Unidos y México, y exhibe una variación geográfica sustancial en su morfología y comportamiento. Este taxón representa un complejo politípico de especies que incluye tres grupos distintos (*californica*, *woodhouseii* y *sumichrasti*) que están asociados con la geografía y que pueden distinguirse de acuerdo a su morfología, genética y comportamiento. Hasta hace poco tiempo, *A. insularis* y *A. coerulescens* se consideraban subespecies dentro del complejo de *A. californica.* Analizamos secuencias de ADN mitocondrial (región de control I) para establecer las relaciones filogenéticas entre los miembros del complejo de *A. californica*, utilizando máxima verosimilitud, inferencia Bayesiana y un método filogenético relajado para construir filogenias. Además, empleamos varios métodos para estimar los tiempos de divergencia entre clados. Nuestros datos revelan que el grupo del interior *woodhouseii* (que contiene el subclado *sumichrasti*) y el grupo costero *californica* han tenido historias evolutivas independientes por un largo período. Sin embargo, las muestras provenientes de una zona de contacto del oeste de Nevada correspondieron genéticamente a *woodhouseii* y morfológicamente a *californica*. *Aphelocoma insularis* evolucionó a partir de la forma costera *californica*, lo que hace que *A. californica* sea un grupo parafilético con respecto a *A. insularis*. Para solucionar el problema de la parafilia y para delinear con mayor exactitud la diversidad que existe dentro de *A. californica*, sugerimos separar este taxón en dos o tres especies: *A. californica*, *A. woodhouseii* y, potencialmente, *A. sumichrasti*.

THE JAYS OF the genus *Aphelocoma* are a highly diverse group that exhibits dramatic geographic variation in ecology and social behavior. All species in the genus are gregarious and most are highly social; however, their breeding systems range from monogamy to cooperative breeding (Brown 1970, Woolfenden and Fitzpatrick 1984, Burt and Peterson 1993, Peterson and Vargas-Barajas 1993, Collins and Corey 1994, Carmen 2004, Watson 2005). The species of *Aphelocoma* inhabit diverse ecological niches ranging from cloud forest, pine forest, and oak woodlands to deserts and even mangroves (Peterson and Vargas-Barajas 1993). Western Scrub-Jays (*A. californica*) range

[1]Present address: Institute of the Environment, University of California, La Kretz Hall, Suite 300, Box 951496, Los Angeles, California 90095, USA. E-mail: ksemple@ucla.edu

The Auk, Vol. 125, Number 4, pages 839–849. ISSN 0004-8038, electronic **ISSN 1938-4254.** **DOI: 10.1525/auk.2008.07088**

from northern Oregon to southern Mexico and from central Texas to the west coast of the United States. Three taxonomic groups have been described within this wide-ranging species based on distinct morphological, ecological, and behavioral differences (Pitelka 1951; Peterson 1992, 1993; Peterson and Vargas-Barajas 1993; Curry et al. 2002). In addition, genetic differences based on allozymes and mitochondrial DNA (mtDNA; ND2 sequences) suggest that there are several distinct clades within Western Scrub-Jays (Peterson 1992, Rice et al. 2003). These clades have been recognized as "groups" by the American Ornithologists' Union (1995) and include the *californica* group from Oregon, California, and the Baja California peninsula in Mexico, the *woodhouseii* group from the interior of the species' range to central Mexico, and the *sumichrasti* group from southern Mexico. In the past, scrub-jays in the *woodhouseii* and *californica* groups were considered separate species (Swarth 1918). The Western Scrub-Jay's closest relatives appear to be the Island Scrub-Jay (*A. insularis*) and the Florida Scrub-Jay (*A. coerulescens*) (Pitelka 1951, Peterson 1992, Saunders and Edwards 2000, Rice et al. 2003, Bonaccorso and Peterson 2007). However, a few of these studies assessed relationships within *Aphelocoma* using two or three individuals from each putative species (Saunders and Edwards 2000, Rice et al. 2003, Bonaccorso and Peterson 2007). Such small sample sizes in a molecular phylogeny do not allow for assessment of intraspecific variation (Irwin 2002, Funk and Omland 2003); thus, the evolutionary relationship of species within the genus remains unclear.

Reconstructing the historical distribution and ecological niche of the Western Scrub-Jay has been the focus of several studies (Rice et al. 2003, Peterson et al. 2004). Peterson et al.'s (2004) study used Pleistocene climate reconstructions and modeled ecological niches of *Aphelocoma* spp. to provide a quantitative hypothesis of the current and Pleistocene distribution of the group. Peterson et al. found that the species distribution was more stable than expected through the Late Pleistocene glaciation and suggested that current genetic differentiation between groups must have predated this period.

There are significant morphological differences between the groups of Western Scrub-Jays (Fig. 1). Birds in the *californica*

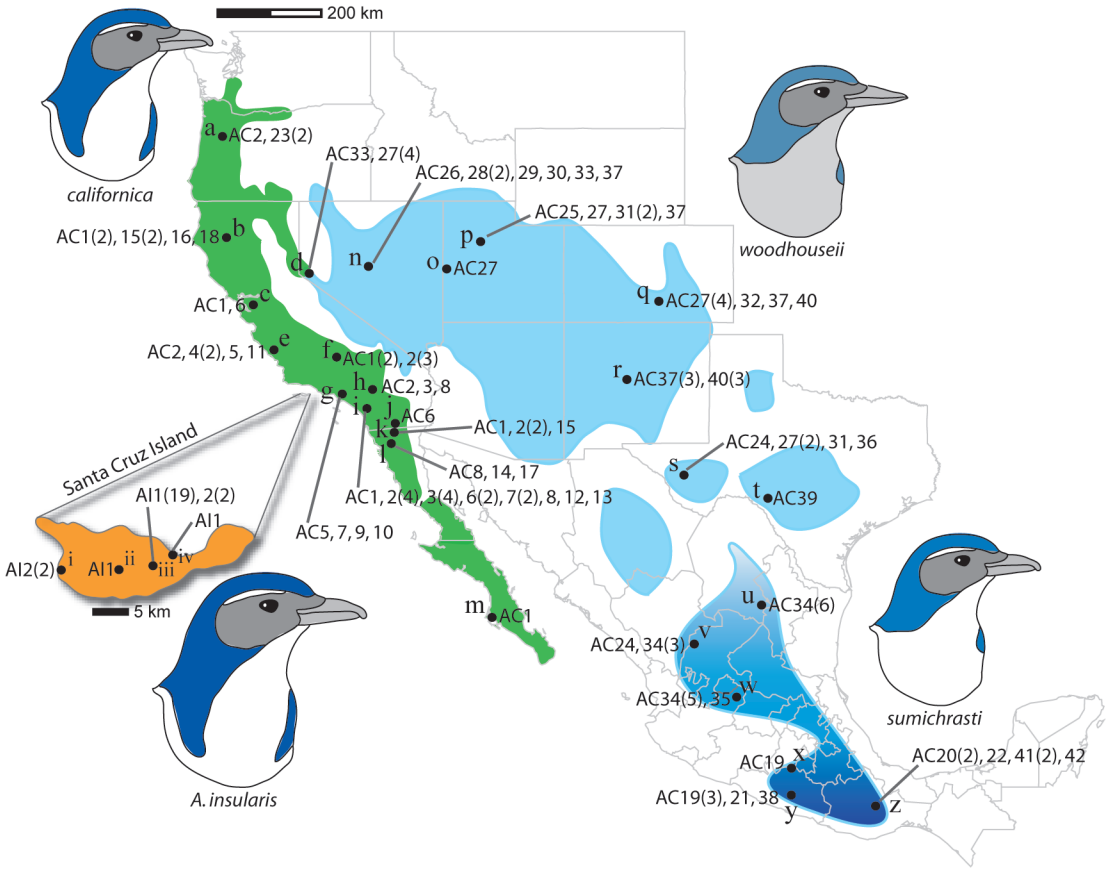

FIG. 1. Distribution of Island and Western scrub-jays, with associated geographic trends in morphological characteristics. Species distributions are adapted from Curry et al. (2002); orange = *A. insularis*, green = *californica*, light blue = *woodhouseii*, and dark blue = *sumichrasti*. Circles indicate sample locations, with associated haplotypes and the number of samples shown in parentheses. Bill-shape differences: hooked vs. pointed; plumage differences: color and presence of a collar. Sample location names are (a) Deschutes; (b) Trinity; (c) Contra Costa; (d) Douglas; (e) Monterey; (f) Kern; (g) Los Angeles; (h) San Bernardino; (i) Orange; (j) Imperial; (k) La Rumorosa, Baja Norte; (l) La Rosa de Castilla, Baja Norte; (m) Baja Sur; (n) Lander; (o) Millard; (p) Tooele; (q) Huerfano; (r) Valencia; (s) Jeff Davis; (t) Val Verde; (u) Coahuila; (v) Zacatecas; (w) Jalisco; (x) Taxco, Guerrero; (y) Xocomanatlan, Guerrero; and (z) Oaxaca. Santa Cruz Island sample location names are (i) Christy, (ii) Pines, (iii) Central Valley, and (iv) Prisoner's Harbor.

group have brighter blue plumage and are generally bolder in behavior than birds in the *woodhouseii* group (Pitelka 1951, Dunn and Garrett 2001, Curry et al. 2002). Bill morphology differs between the two groups and reflects differences in diet associated with habitat (Peterson 1993, Bardwell et al. 2001). Scrub-jays are not restricted to a specific habitat type, but generally *californica* jays occur in oak woodland habitat and *woodhouseii* jays occur in pinyon–juniper woodland (Pitelka 1951, Curry et al. 2002). There is also a south-to-north cline of increasing body size in the *californica* group, whereby birds in the northern part of the range are larger. By contrast, a north-to-south cline exists in the *woodhouseii* group, with birds in southern Mexico that belong to the *sumichrasti* group being the largest of any Western Scrub-Jay (Pitelka 1951, Peterson and Vargas-Barajas 1993, Curry et al. 2002).

The *woodhouseii* and *californica* groups are not sympatric throughout most of their range, being mostly separated by desert barriers. However, there are several areas in the western United States where the groups have been shown to come into contact. They are found within 20 km and 100 km of each other in the Owens Valley in central California and in the Mojave Desert of southern California, respectively (Pitelka 1951, Curry et al. 2002). In addition, there is a small contact zone in western Nevada for two Western Scrub-Jay subspecies (*A. c. oocleptica* [formerly *A. c. superciliosa*] and *A. c. nevadae*; Pitelka 1951). In this contact zone, there is an abrupt intergradation between *woodhouseii* (*A. c. nevadae*) and *californica* (*A. c. oocleptica*) across a narrow swath of continuous pinyon–juniper woodlands. Migrants can readily be detected by phenotypic differences (Pitelka 1951). A study examining phenotypic characters of >2,600 museum skins from throughout the western United States showed very low rates of immigration to the *woodhouseii* group but slightly higher levels to the *californica* group, particularly in central and southern California (Peterson 1991).

Until 1995, when the species were split into three, the Western, Island, and Florida scrub-jays were considered one polytypic species (*A. coerulescens*; American Ornithologists' Union 1995). Previous taxonomic designations provided an unbalanced view of the diversity within this clade, and it has been argued that the species should either remain one polytypic species or be split into five (Dunn and Garrett 2001, Curry et al. 2002, Watson 2005). Under this scheme, the current Island and Florida scrub-jay designations would remain recognized and the "groups" within Western Scrub-Jays would be given full species status.

Here, we explore the phylogenetic relationships of the Western Scrub-Jay groups and their relationship to Island Scrub-Jays by sequencing the control region I of mtDNA. We have included samples of Western Scrub-Jays from throughout their geographic range. In constructing phylogenetic trees using several methods, our goal was to determine whether there was a high level of genetic divergence associated with the large morphological and behavioral divergence within Western Scrub-Jays. We found evidence for two, and possibly three, distinct lineages within Western Scrub-Jays and argue that the current taxonomic treatment of scrub-jays does not reflect this divergence. Our results support the division of three "groups" that should be designated as three species: California Scrub-Jay (*A. californica*), Woodhouse's Scrub-Jay (*A. woodhouseii*), and Sumichrast's Scrub-Jay (*A. sumichrasti*).

Methods

Sample collection.—We obtained Western Scrub-Jay tissue samples from the Chicago Field Museum, originally collected by A. Townsend Peterson. Western Scrub-Jays were sampled throughout their range in the United States and Mexico (Fig. 1). Sequences obtained from these samples were added to those previously obtained from Island and Western scrub-jays (Delaney and Wayne 2005).

Mitochondrial DNA sequencing.—We extracted genomic DNA from tissue with the Qiagen DNA mini kit (Qiagen, Valencia, California). The DNA samples were stored in TE buffer (10mM Tris-Cl pH 8.0, 1mM EDTA pH 8.0) at –20°C. We used the primers JCR03 and H1248 to amplify the entire mtDNA control region through polymerase chain reaction (PCR; Tarr 1995, Saunders and Edwards 2000). The control region I is rapidly evolving and is an appropriate marker for population-level phylogeographic analysis (Avise 2000). After agarose gel purification of amplified products with the Ultraclean 15 DNA Purification Kit (Mo Bio Laboratories, Carlsbad, California), we performed cycle sequencing with the JCR03 primer to sequence control region I (GenBank accession numbers for AC1–AC42 are EU490619–EU490660, and for AI1–AI2 are EU490661–490662). An ABI 3700 capillary sequencer was used for automated sequencing.

Analysis of mtDNA sequences.—We aligned control region I sequences with SEQUENCHER, version 3.1.1 (Genecodes, Ann Arbor, Michigan), and used COLLAPSE, version 1.2 (Posada 2004), to eliminate redundant haplotypes. To determine the best model of nucleotide substitution for our sequences, we used a hierarchical likelihood ratio test in MRMODELTEST, version 2.2 (Nylander 2004). The HKY model of nucleotide substitution with gamma-distributed rate heterogeneity among sites (HKY+G) was the best model for our sequences.

We used three approaches to reconstruct the phylogenetic relationships of control-region sequences. First, we used the maximum-likelihood criterion, the HKY+G model, and 100 bootstrap replications to create a phylogram with bootstrap support in TREEFINDER (Jobb 2007). Second, we used the HKY+G model in MRBAYES, version 3.1.2, for 1 million Markov-chain Monte Carlo (MCMC) generations, sampling every 1,000 generations. We then generated a 50% majority-rule consensus tree in PAUP*, version 4.0 (Swofford 1998), for the 20,000 trees that resulted from the two independent MRBAYES runs (Ronquist and Huelsenbeck 2003). Third, we used the program BEAST (Bayesian evolutionary analysis sampling trees) for a Bayesian MCMC relaxed phylogenetic approach, which allows for the rates of evolution among branches of the tree to vary (Drummond et al. 2002, 2006; Drummond and Rambaut 2006). In BEAST, we assumed an HKY+G model and rates of evolution on each branch of the phylogeny were set as uncorrelated, and we assumed that the rate of each branch was independently drawn from a log normal distribution (UCLN; Drummond and Rambaut 2006, Drummond et al. 2006). We assumed a mean substitution rate of 1.04×10.00^{-7} substitutions site^{-1} year^{-1} as a prior in the Bayesian BEAST runs (Avise 1994, Ho et al. 2005). Assuming a constant population size and using the UCLN criteria, two independent MCMC runs of 10 million steps were combined to obtain an estimate of the posterior distribution of parameters of interest in the phylogeny.

We used a program complementary to BEAST, LOGCOMBINER, to combine the results of the two runs and a second complementary program, TREEANNOTATOR, to create a consensus tree from the 20,000 trees generated in two runs (Drummond and Rambaut 2006).

We estimated sequence diversity as the number of haplotypes (*h*), haplotype diversity (*Hd*), nucleotide diversity (π), and the average number of nucleotide differences (*k*) within each group (*californica*, *woodhouseii*, *sumichrasti*, and *A. insularis*) using DNASP, version 4.0 (Rozas and Rozas 1999). Using an analysis of molecular variance (AMOVA), we calculated ϕ_{CT} among groups, ϕ_{ST} among sampling locations among groups, and ϕ_{SC} among sampling locations within groups with ARLEQUIN, version 2.0 (Schneider et al. 2000), assuming the Tamura-Nei nucleotide substitution model (Tamura and Nei 1993) with a gamma parameter and 1,000 permutations for significance. In ARLEQUIN, we also generated a minimum spanning tree using absolute pairwise differences to reconstruct the relationship between haplotypes. Pairwise sequence divergence between groups was estimated using MEGA, version 3.1 (Kumar et al. 2001). We used the Tamura-Nei model with a gamma parameter to calculate average and net distances between clades. Standard errors were estimated by performing 500 bootstrap replicates. Net divergence measures take into account differences between sequences within the same clade (Nei 1987). The Tamura-Nei model was used for this analysis because the software does not have the HKY+G model. However, HKY is very similar to the Tamura-Nei model, except that in addition to accounting for transition–transversion and GC content biases, the Tamura-Nei distance also accounts for purine–pyrimidine transitions. The HKY distance is a closely related special case of the Tamura-Nei distance; therefore, it is appropriate to use either measure on control-region sequences, especially for closely related species (Tamura and Nei 1993, Nei and Kumar 2000).

We used two methods for assessing variation in evolutionary rate among lineages. First, we compared the relative evolutionary rates of scrub-jay lineages using Tajima's test in MEGA (Kumar et al. 2001). Tajima's relative rate test compares two sequences from different lineages to each other and an outgroup or third lineage (Tajima 1993). We rejected the molecular-clock hypothesis if the expected number of nucleotide substitutions per site was not similar between the focal lineages and the outgroup. If Tajima's test was not significant, we could not reject the molecular-clock hypothesis and assumed that lineages were evolving at similar rates. Second, we tested for lineage substitution-rate heterogeneity using the Bayesian relaxed phylogenetic approach in BEAST. The data set was run under the UCLN model, and the coefficient of variation was estimated. The UCLN model samples the branch-rate distribution as the coefficient of variation of branch rates, which can be used to determine whether a data set is "clock-like" (Drummond et al. 2006). The coefficient of variation (σ) ranges from 0 to 1, where 0 is perfectly clock-like.

We used two methods to estimate divergence time between lineages. First, we used a strict molecular clock with the net percent sequence divergence and divergence rate of 0.208 substitutions site^{-1} Ma^{-1} for control region I, which corresponds to ~20% divergence Ma^{-1} (Delaney and Wayne 2005). We believe that this rate gives a conservative (i.e., shorter) estimate of divergence time and is in the middle of a range of published rates for this marker (0.02 substitutions site^{-1} Ma^{-1} [Quinn 1992] to 0.93 substitutions site^{-1} Ma^{-1} [Lambert et al. 2002]). Second, relaxed phylogenetic methods permit estimation of divergence times despite uncertainty about evolutionary rates among clades and calibration times because of the lack of a fossil record in this group (Peterson 1992). On the basis of a divergence rate of 20% Ma^{-1}, we used a mean substitution rate of 1.4×10^{-7} substitutions site^{-1} year^{-1} as a prior in the BEAST phylogenetic analysis to calculate divergence time in years.

Results

We sequenced 389 base pairs (bp) of control region I for 82 Western Scrub-Jays and combined these sequences with those from 36 Western Scrub-Jays from California and 25 Island Scrub-Jays from Santa Cruz Island (Delaney and Wayne 2005), for a total of 143 sequences. We found 44 haplotypes (42 Western Scrub-Jay haplotypes and 2 Island Scrub-Jay haplotypes; Fig. 1). We also obtained homologous control-region I sequences from GenBank for Mexican Jay (*A. ultramarina*), Florida Scrub-Jay, and Unicolored Jay (*A. unicolor*) that were used as outgroups.

Phylogenetic relationships.—We identified two major clades within Western Scrub-Jays (Figs. 2 and 3). These two Western Scrub-Jay clades did not share any haplotypes with each other or with Island Scrub-Jays (Figs. 2 and 3). One clade included all the samples on the west coast of the United States and Baja California, Mexico; following Pitelka (1951), we refer to this clade as the "*californica* group." Within this clade, two further groups were well supported and appeared to be associated with geography (Figs. 2 and 3). First, two haplotypes were found only in southern California samples (AC3 and AC13). Second, two haplotypes were found only in samples from the northern part of *californica's* range (AC23 and AC4). A second clade describing the individuals sampled from the interior United States and mainland Mexico corresponds to Pitelka's (1951) *woodhouseii–sumichrasti* group. Within this major clade was a grouping that comprised individuals from Oaxaca and Guerrero in southern Mexico and that corresponds to Pitelka's (1951) *sumichrasti* group. The *sumichrasti* clade was not well supported with maximum-likelihood or Bayesian methods; however, a clade consisting of four haplotypes from the *sumichrasti* group was well supported (Figs. 2 and 3; AC19–AC22). Some geographic structure was evident in the *woodhouseii* group, in that there were two common haplotypes, AC27 and AC34, restricted to the Great Basin and Mexico, respectively (Fig. 4). Within the *woodhouseii* clade, we found five individuals representing two haplotypes (AC27 and AC33) from the narrow contact zone in western Nevada that were identified by plumage as *A. c. oocleptica*, part of the *californica* group. Although this clade comprised birds from both the *woodhouseii* and *californica* groups, we designated it the "*woodhouseii* group" and assumed that the birds from western Nevada represented a possible example of introgression or incomplete lineage-sorting (see below). Tree topologies differed with regard to the *woodhouseii–sumichrasti* clade. The clade was well supported with the relaxed phylogenetic method (99%; Fig. 3). However, in the maximum-likelihood tree one haplotype, AC39 from southern Texas, was basal to all clades and there was low bootstrap (<50%) and Bayesian (68%) support for the clade containing the rest of the *woodhouseii* haplotypes (Fig. 2).

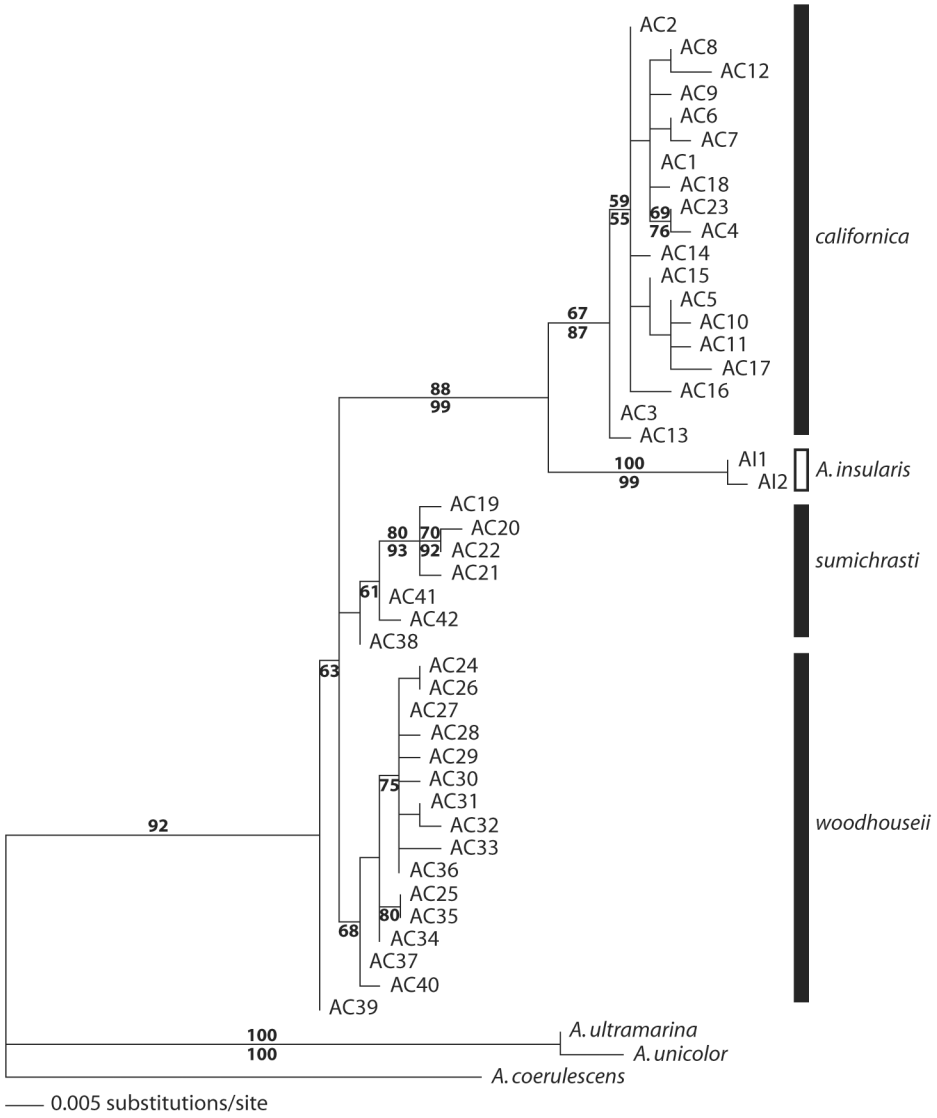

FIG. 2. Maximum-likelihood tree of Western (black boxes) and Island (white box) scrub-jay haplotypes. Maximum-likelihood bootstrap values are above branches, and posterior probabilities from MRBAYES runs are below branches. Bootstrap values <50% are not shown.

Absolute pairwise distances from ARLEQUIN were used to generate a minimum spanning tree, which portrayed the number of substitutions between haplotypes (Fig. 4). A 1-bp deletion was common to all of the samples within the *woodhouseii* clade, including the *A. c. oocleptica* birds from western Nevada (Fig. 4). The minimum spanning tree showed that there were no haplotypes shared between the two major Western Scrub-Jay clades, or between *woodhouseii* and *sumichrasti* (Fig. 4).

Genetic variability and divergence.—We assessed nucleotide and haplotype diversity of the three Western Scrub-Jay groups separately (Table 1A). Nucleotide diversity was similar between all the groups, whereas *Hd* was larger in *californica* samples and *k* was smaller in the *woodhouseii* samples. Island Scrub-Jay measures of sequence diversity were an order of magnitude lower than those of any other group (Delaney and Wayne 2005).

Net sequence divergence between clades varied between 1.0% and 3.8%. The greatest divergence occurred between *californica* and *A. insularis* (3.8 ± 1.2% [SE]). There was also large divergence (3.2 ± 1.1%) between the two primary clades *californica–insularis* versus *woodhouseii–sumichrasti*. A smaller divergence (1.00 ± 0.05%) was found between *woodhouseii* and *sumichrasti*. The average sequence divergence was higher between groups than within groups (Table 1B). An AMOVA analysis revealed an overall ϕ_{ST} of 0.86 ($P < 0.0001$), $\phi_{CT} = 0.90$ ($P < 0.0001$), and $\phi_{SC} = 0.22$ ($P < 0.0001$), which indicates that most of the variation was among groups. Pairwise ϕ_{ST} values between groups were large and highly significant ($P < 0.0001$; Table 1B).

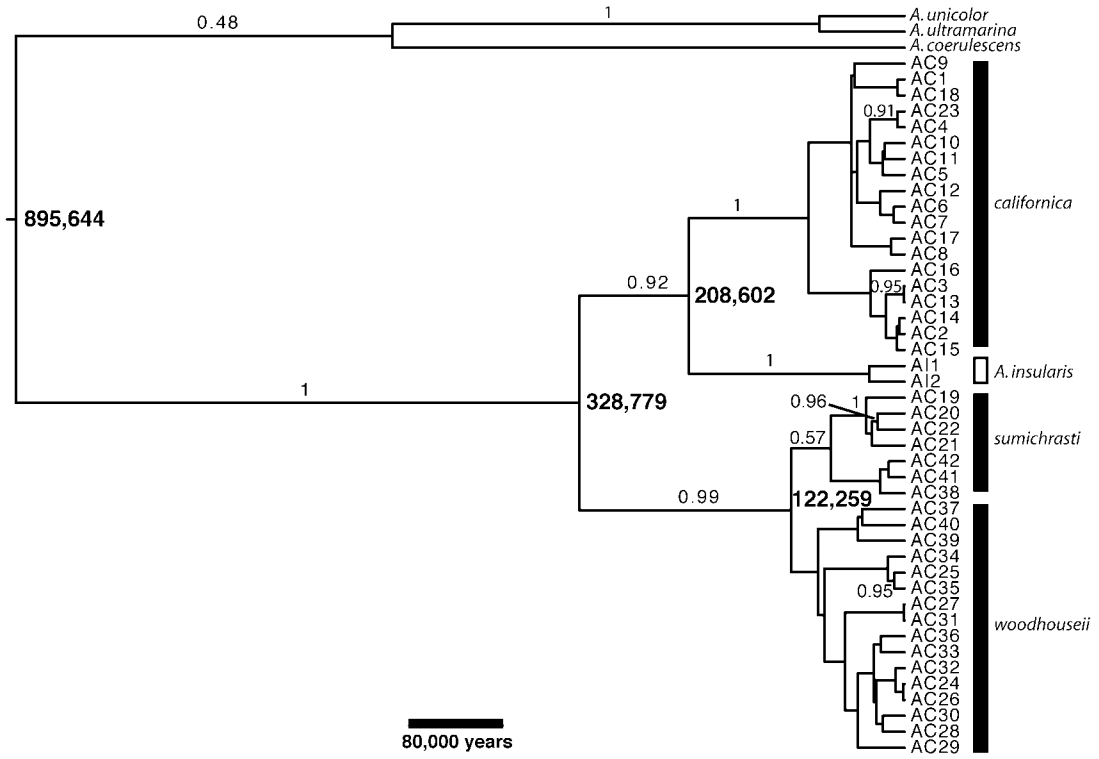

FIG. 3. BEAST tree of Western (black boxes) and Island (white box) scrub-jay haplotypes, with posterior probabilities on branches and divergence-time estimates in years (95% HPD presented in text) at nodes.

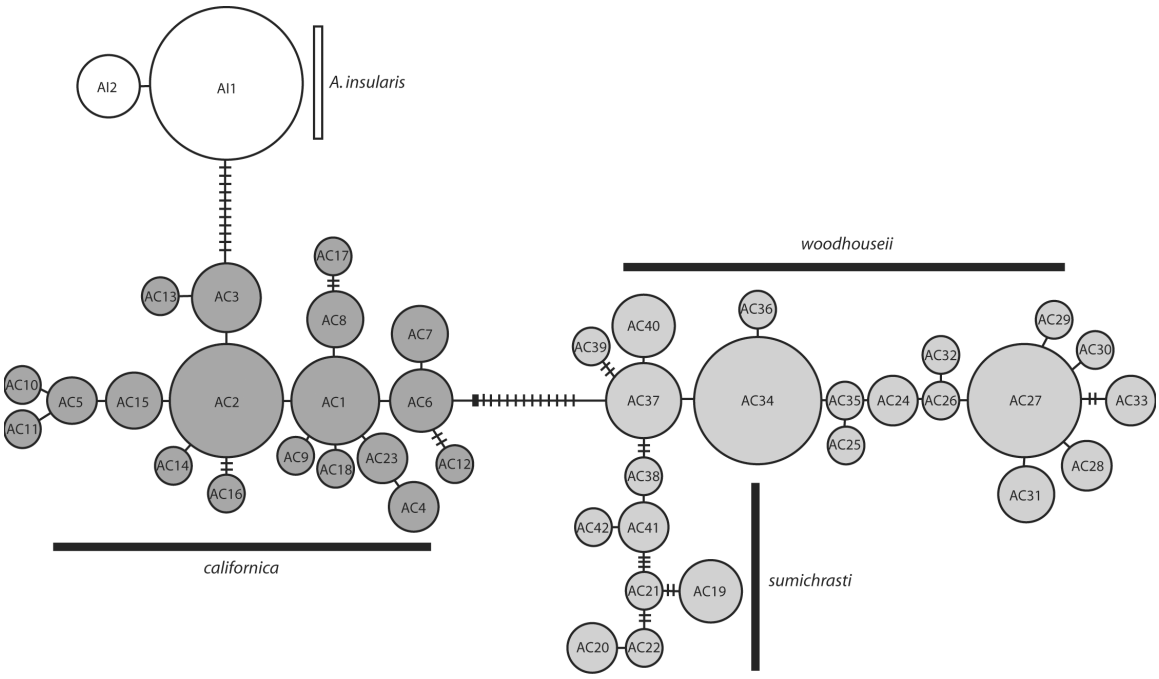

FIG. 4. Haplotype network tree of Western and Island scrub-jays. The number of absolute pairwise differences is shown on each branch connecting haplotypes; sizes of circles indicate the relative number of sequences. One insertion–deletion, represented by a black rectangle, was found between *californica–A. insularis* and *woodhouseii–sumichrasti*.

TABLE 1. Mitochondrial sequence divergence between groups and diversity within groups. (A) Sequence diversity (n = number of sequences, h = number of haplotypes, Hd = haplotype diversity, π = nucleotide diversity, and k = average number of nucleotide differences). (B) Pairwise sequence divergence between groups using the Tamura-Nei model of evolution and 500 bootstrap replicates for standard errors (SE). Above diagonal is the average divergence between groups (± SE), diagonal elements are the average divergence within groups (± SE), and below the diagonal is the ϕ_{ST} between groups.

A	Group	n	h	Hd	π	k
	californica	54	20	0.915	0.00652	2.512
	woodhouseii	53	13	0.856	0.00638	1.684
	sumichrasti	12	7	0.879	0.00704	2.667
	A. insularis	25	2	0.28	0.00072	0.28

B	Group	*californica*	*woodhouseii*	*sumichrasti*	*A. insularis*
	californica	0.006 ± 0.002	0.042 ± 0.013	0.047 ± 0.014	0.041 ± 0.014
	woodhouseii	0.83	0.005 ± 0.002	0.016 ± 0.006	0.056 ± 0.017
	sumichrasti	0.88	0.50	0.008 ± 1.630	0.054 ± 0.017
	A. insularis	0.90	0.89	0.95	0.001 ± 0.001

Molecular-clock estimates of divergence time between clades.—We tested for differences in rates of nucleotide substitution because substitution-rate heterogeneity can lead to errors when calculating clade divergence times or phylogenetic relationships. Two methods were used to test for substitution-rate heterogeneity between clades within Western Scrub-Jays. First, we compared sequences from our three Western Scrub-Jay groups and Island Scrub Jays with the other *Aphelocoma* sequences as outgroups for Tajima's (1993) test and found that there were no significant differences in the rates of evolution in any scrub-jay lineage (Tajima's test, $P > 0.05$ in all comparisons). Second, we analyzed our entire data set with BEAST to estimate the coefficient of variation in rates. We found that the coefficient of variation was small ($\sigma = 0.273$; 95% highest posteror density [HPD]: $[4.857 \times 10^{-4}] - 0.651$).

Divergence-time estimates.—Because we found no significant differences with our Tajima's test, we first used a molecular clock to estimate divergence times between lineages based on net sequence divergence and a divergence rate of 0.208 substitutions site^{-1} Ma^{-1} (Klicka and Zink 1997, Lovette et al. 2002, Lovette 2004). Divergence time between the coastal and interior clades was 153,846 ± 52,884 years. Divergence time between the *woodhouseii* and *sumichrasti* clades was 48,076 ± 2,403 years. The previously published divergence time between *californica* and *A. insularis* was 150,961 ± 4,327 years (Delaney and Wayne 2005). Our BEAST analysis estimated that the divergence time between the interior and coastal clades was 328,779 years (95% HPD: 195,154–496,972). Divergence time between the *woodhouseii* and *sumichrasti* clades was 122,259 years (95% HPD: 56,636–197,009), and that between *californica* and *A. insularis* was 208,602 years (95% HPD: 114,366–325,066). Finally, divergence time between Western and Island scrub-jays and the other *Aphelocoma* spp. was 895,644 years (95% HPD: 483,476–1,354,982; Fig. 3). Consequently, the relative branching time between coastal and interior clades was 50–100% greater than the divergence between other groups.

DISCUSSION

We found substantial genetic divergence within the Western Scrub-Jay, which suggests a long history of isolation and evolutionary divergence within this species. Our results show that Western Scrub-Jays comprise two primary clades, the interior clade containing a possible *sumichrasti* subclade. There were no shared haplotypes between the two clades (Figs. 2, 3, and 4). The two primary clades correspond to groupings of geographic locations with one clade consisting of birds from California, Oregon, and Baja California, Mexico (*californica* group), and a second clade with birds from the interior United States and interior Mexico (*woodhouseii* group). A third clade that consisted of birds from southern Mexico was not well supported when all the samples from that area were considered (*sumichrasti* group). The *sumichrasti* group was separated from *woodhouseii* by only two substitutions. Between the two major clades, there was an informative insertion–deletion that separated the groups. The *A. insularis* clade was well supported in all analyses.

Older haplotypes are predicted to be found at interior nodes within a minimum spanning tree, with higher frequencies and more widely distributed (Posada and Crandall 2001). The most frequent haplotype within *woodhouseii*, AC34, is found only in central Mexico (Fig. 4). However, another common haplotype within this group, AC27, is found widely throughout the Great Basin region. Similarly, in the *californica* group, two haplotypes are widely distributed and have high frequencies (AC1 and AC2; Fig. 4). The placement and frequencies of these haplotypes suggest a long history of isolation and population stability (Posada and Crandall 2001).

Hybridization between *woodhouseii* and *californica* has been previously suggested on the basis of phenotype. Peterson (1991) examined 2,647 museum skins collected from locations in California, Nevada, and northern Mexico and found only 15 individuals that he suggested could be possible hybrids between *californica* and *woodhouseii* on the basis of morphological characters. The five birds from the contact zone in the Pine Nut

Mountains of western Nevada (Douglas County) are interesting in this regard, because they were identified at the time of collection as *A. c. oocleptica* (by A. T. Peterson), yet had haplotypes shared with *woodhouseii* birds. There are several possible explanations for this result. First, the contact zone described by Pitelka (1951) may have shifted, and birds captured there in the late 1980s may actually have been *A. c. nevadae* of the *woodhouseii* group. This seems unlikely, because plumage and morphological differences are readily diagnostic. Second, there could be introgression resulting from recent gene flow between groups. Third, there could be incomplete lineage-sorting, which would suggest a much more ancient hybridization between the two groups. However, it is difficult to distinguish between introgression and incomplete lineage-sorting (Funk and Omland 2003). Regardless of the reason, it appears that we have detected an area where morphology and mtDNA haplotypes are not congruent. Future research will focus on increasing the number of samples from this area and using variable nuclear markers, such as microsatellites, to further characterize this potential contact zone.

Scrub-jays in the *sumichrasti* group are morphologically and behaviorally different from those in the *woodhouseii* group. The only known population of cooperatively breeding Western Scrub-Jays is a *sumichrasti* population in Oaxaca (Burt and Peterson 1993). Approximately 60% of nests within this population were observed to have one to four adult helpers at the nest (Burt and Peterson 1993, Curry et al. 2002). Birds of the *sumichrasti* group are also the largest of the Western Scrub-Jays and have plumage distinguishable from that of *woodhouseii* (Pitelka 1951). Their blue plumage is brighter, and their throat color is white (Pitelka 1951). They also have hooked bills similar to those of *californica* and *A. insularis*, whereas individuals of *woodhouseii* have pointed bills. Despite these behavioral and morphological differences, we found low bootstrap and Bayesian support separating this group from the *woodhouseii* group (Figs. 2 and 3). For the *sumichrasti* group, we found eight haplotypes in 13 individuals; however, bootstrap and Bayesian support were high for only four of those haplotypes (AC19, AC20, AC21, and AC22; Figs. 2 and 3), representing eight individuals. However, the minimum spanning tree shows that the *sumichrasti* group is closely related to the *woodhouseii* clade and that the groups do not share haplotypes. In addition, a significant level of genetic structure was found between *woodhouseii* and *sumichrasti* (Table 1B).

The current taxonomic treatment of scrub-jays does not reflect the distinction of the lineages we found here. Several authors have advocated the taxonomic treatment of the scrub-jay species complex as either one highly polytypic species or five distinct species (Dunn and Garrett 2001, Curry et al. 2002). In a similar example, the polytypic "blue grouse" (*Dendragapus obscurus*) was recently recognized as two species, Dusky Grouse (*D. obscurus*) and Sooty Grouse (*D. fuliginosis*), on the basis of an mtDNA phylogeny along with morphological and behavioral evidence (Barrowclough et al. 2004, Banks et al. 2006). If the current taxonomy is retained, Western Scrub-Jays are paraphyletic with respect to Island Scrub-Jays. The reclassification of Western Scrub-Jays into at least two, and potentially three, distinct species would more accurately reflect the morphological, ecological, behavioral, and genetic differences among these groups. Island Scrub-Jays and Florida Scrub-Jays were officially recognized as full species on the basis of morphology and genetic distinctiveness (American Ornithologists' Union 1995). A new taxonomic designation would solve the problem of paraphyly for Western Scrub-Jays in relation to Island Scrub-Jays. Divergence time between *A. insularis* and *californica* was ~200,000 years. Divergence time between *californica* and *woodhouseii* was 1.5× that estimation, yet the groups remain lumped within Western Scrub-Jays. Consequently, we argue that Western Scrub-Jays should be separated into at least two species: California Scrub-Jay and Woodhouse's Scrub-Jay. More tentatively, Sumichrast's Scrub-Jay may be a distinct species within the latter group. However, given the lack of statistical support for the isolation of the *sumichrasti* lineage and the apparent introgression or incomplete lineage sorting for the birds in western Nevada, it may be suggested that taxonomic revision should await further studies. The high mutation rate of the control region I is best for assessing phylogenetic structure with more recent divergence times (Avise 2000); therefore, using additional molecular markers may increase support for phylogenetic relationships. In addition, there are areas within the Western Scrub-Jay distribution that were not sampled. Increasing the number of samples per location and including more sample locations may help to identify additional rare haplotypes (e.g., AC39) and species boundaries. Despite these caveats, we believe that the concordant genetic, behavioral, and morphological evidence suggests that a change in taxonomy is warranted.

A previous study with allozymes found large differences in the rates of evolution between lineages (Peterson 1992). Evolutionary-rate heterogeneity among lineages within a phylogeny can affect the interpretation of divergence times between groups. We found that there were no significant differences in evolutionary rate between scrub-jay lineages by using a Tajima's test. Our relaxed phylogenetic approach using control-region sequences suggested that the branches may be evolving in a relatively clock-like manner, with a coefficient of variation smaller than others reported in similar studies (0.32 for marsupials to 0.51 for viruses; Drummond et al. 2006). Because we found a small amount of rate variation among lineages, we believe that our divergence-time estimates from the BEAST analysis are more robust. The BEAST estimate of divergence time between interior and coastal lineages was >325,000 years. Our BEAST estimation of the divergence time between *californica* and *A. insularis* was higher than our previous estimation based on a simple molecular clock; however, it was within the error range of our past molecular-clock calculations (Delaney and Wayne 2005).

The divergence-time estimate of >325,000 years between the *californica–insularis* and *woodhouseii–sumichrasti* lineages indicates that this split happened during the Late Pleistocene. Comparative studies have shown that many avian taxa have diversified and speciated because of vicariance caused by glacial advances in the Pleistocene and Pliocene, though there is debate about the relative importance of events in each epoch (Klicka and Zink 1997, Johnson and Cicero 2004, Weir and Schluter 2004, Zink et al. 2004). An examination of past suitable habitat distribution for scrub-jays indicated that the present-day distribution of Western Scrub-Jays is similar to that of 21,000 years ago (Peterson et al. 2004). However, California and the Great Basin woodlands have had separate evolutionary histories since the late Pliocene

or early Pleistocene (Axelrod 1973). In addition to habitat differences, there are climatic differences that have been proposed as factors affecting the divergence of several species in the western United States. First, Oak Titmouse (*Baeolophus inornatus*) and Juniper Titmouse (*B. ridgwayi*) are sister species with geographic ranges similar to those of *californica* and *woodhouseii*, respectively. When 15 temperature and precipitation variables were compared between geographic ranges of Oak and Juniper titmice, 14 of the 15 were significantly different, which suggests a barrier to sympatry that would maintain genetic divergence between these closely related species (Cicero 2004). Second, Yellow-billed Magpies (*Pica nuttalli*) are found only in California, whereas Black-billed Magpies (*P. hudsonia*) are found throughout the Great Basin and Alaska. Yellow-billed Magpies are more heat-tolerant than Black-billed Magpies, which suggests that they have physiologically adapted to the warmer Mediterranean climate of California (Hayworth and Weathers 1984). The long isolation and divergent climate of woodland habitats separated by a large vicariant barrier such as the Sierra Nevada Mountains would provide an opportunity for adaptive and genetic divergence in Western Scrub-Jays.

The phylogeographic break that exists between *woodhouseii* and *californica* corresponds to a recognized contact-zone "hotspot" (Johnson 1978, Hewitt 2000). A contact-zone hotspot is a geographic area where multiple hybrid zones, phylogeographic breaks, or contact zones, or some combination of these, are significantly clustered. This recognized hotspot extends from southern Oregon south to the Sierra Nevada Mountains that separate Nevada and California. In addition, many east–west mtDNA phylogeographic divisions have been identified in California along the Sierra Nevada and farther south (Cicero and Johnson 1992, Johnson and Marten 1992, Calsbeek et al. 2003). Interestingly, although the scrub-jay data correspond to the east–west division over the Sierra Nevada, these studies found that hotspots and phylogeographic splits for birds were not as strong as those for other species, presumably because of higher avian dispersal abilities. Scrub-jays hold year-round territories (Carmen 1988, 2004; Curry et al. 2002) and are relatively weak fliers (Pitelka 1951), so it is possible that more sedentary birds will match phylogeographic patterns of less vagile animals, such as lizards and arthropods.

Molecular studies of broadly distributed organisms are revealing large phylogenetic divisions in North American vertebrates (e.g., Klicka and Zink 1997, Johnson and Cicero 2004). By examining such phylogeographic patterns, the history of speciation in North American birds can be revealed and evolutionary hypotheses explored. Jays in the genus *Aphelocoma* are emblematic species for the study of ecological adaptations (Peterson 1993, Peterson and Vargas-Barajas 1993) and the evolution of social behavior (Brown 1970, 1987; Peterson and Burt 1992). The present study, combined with future robust genus-level phylogenies, may provide the necessary historical context needed to reconstruct the origins, speciation history, and evolution of sociality in this group.

Acknowledgments

We thank D. Willard of the Field Museum of Natural History, Chicago, and J. McCormack and A. T. Peterson for helpful comments on the manuscript.

Literature Cited

American Ornithologists' Union. 1995. Fortieth supplement to the American Ornithologists' Union *Check-list of North American Birds*. Auk 112:819–830.

Avise, J. C. 1994. Molecular Markers, Natural History and Evolution. Chapman & Hall, New York.

Avise, J. C. 2000. Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, Massachusetts.

Axelrod, D. I. 1973. History of the Mediterranean ecosystem in California. Pages 225–277 *in* Mediterranean Type Ecosystems: Origin and Structure. (F. di Castri and H. A. Mooney, Eds.). Springer-Verlag, New York.

Banks, R. C., C. Cicero, J. L. Dunn, A. W. Kratter, P. C. Rasmussen, J. V. Remsen, Jr., J. D. Rising, and D. F. Stotz. 2006. Forty-seventh supplement to the American Ornithologists' Union *Check-list of North American Birds*. Auk 123: 926–936.

Bardwell, E., C. W. Benkman, and W. R. Gould. 2001. Adaptive geographic variation in Western Scrub-Jays. Ecology 82: 2617–2627.

Barrowclough, G. F., J. G. Groth, L. A. Mertz, and R. J. Gutiérrez. 2004. Phylogeographic structure, gene flow and species status in Blue Grouse (*Dendragapus obscurus*). Molecular Ecology 13:1911–1922.

Bonaccorso, E., and A. T. Peterson. 2007. A multilocus phylogeny of New World jay genera. Molecular Phylogenetics and Evolution 42:467–476.

Brown, J. L. 1970. Cooperative breeding and altruistic behaviour in the Mexican Jay, *Aphelocoma ultramarina*. Animal Behaviour 18:366–378.

Brown, J. L. 1987. Helping and Communal Breeding in Birds. Princeton University Press, Princeton, New Jersey.

Burt, D. B., and A. T. Peterson. 1993. Biology of cooperative-breeding scrub jays (*Aphelocoma coerulescens*) of Oaxaca, Mexico. Auk 110:207–214.

Calsbeek, R., J. N. Thompson, and J. E. Richardson. 2003. Patterns of molecular evolution and diversification in a biodiversity hotspot: The California Floristic Province. Molecular Ecology 12: 1021–1029.

Carmen, W. J. 1988. Behavioral ecology of the California scrub jay (*Aphelocoma coerulescens californica*): A noncooperative breeder with close cooperative relatives. Ph.D. dissertation, University of California, Berkeley.

Carmen, W. J. 2004. Noncooperative breeding in the California Scrub-Jay. Studies in Avian Biology, no. 28.

Cicero, C. 2004. Barriers to sympatry between avian sibling species (Paridae: *Baeolophus*) in local secondary contact. Evolution 58:1573–1587.

Cicero, C., and N. K. Johnson. 1992. Genetic differentiation between populations of Hutton's Vireo (Aves: Vireonidae) in disjunct allopatry. Southwestern Naturalist 37:344–348.

Collins, C. T., and K. A. Corey. 1994. Delayed breeding in the Santa Cruz Island Scrub-Jay: Why not be cooperative? Pages 371–378 *in* The Fourth California Islands Symposium: Update on the Status of Resources (W. L. Halverson and G. J. Maender, Eds.). Santa Barbara Museum of Natural History, Santa Barbara, California.

Curry, R. L., A. T. Peterson, and T. A. Langen. 2002. Western Scrub-Jay (*Aphelocoma californica*). *In* The Birds of North America, no. 712 (A. Poole and F. Gill, Eds.). Birds of North America, Philadelphia.

Delaney, K. S., and R. K. Wayne. 2005. Adaptive units for conservation: Population distinction and historic extinctions in the Island Scrub-Jay. Conservation Biology 19:523–533.

Drummond, A. J., S. Y. W. Ho, M. J. Phillips, and A. Rambaut. 2006. Relaxed phylogenetics and dating with confidence. PloS Biology 4:699–710.

Drummond, A. J., G. K. Nicholls, A. G. Rodrigo, and W. Solomon. 2002. Estimating mutation parameters, population history and genealogy simultaneously from temporally spaced sequence data. Genetics 161:1307–1320.

Drummond, A. J., and A. Rambaut. 2006. BEAST, version 1.4. [Online.] Available at beast.bio.ed.ac.uk/.

Dunn, J. L., and K. L. Garrett. 2001. Parapatry in Woodhouse's and California scrub-jays revisited. Western Birds 32: 186–187.

Funk, D. J., and K. E. Omland. 2003. Species-level paraphyly and polyphyly: Frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annual Review of Ecology, Evolution and Systematics 34:397–423.

Hayworth, A. M., and W. W. Weathers. 1984. Temperature regulation and climatic adaptation in Black-billed and Yellow-billed magpies. Condor 86:19–26.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. Nature 405:907–913.

Ho, S. Y. W., M. J. Phillips, A. Cooper, and A. J. Drummond. 2005. Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. Molecular Biology and Evolution 22:1561–1568.

Irwin, D. E. 2002. Phylogeographic breaks without geographic barriers to gene flow. Evolution 56:2383–2394.

Jobb, G. 2007. TREEFINDER. [Online.] Available at www.treefinder.de.

Johnson, N. K. 1978. Patterns of avian geography and speciation in the intermountain region. Great Basin Naturalist Memoirs 2: 137–159.

Johnson, N. K., and C. Cicero. 2004. New mitochondrial DNA data affirm the importance of Pleistocene speciation in North American birds. Evolution 58:1122–1130.

Johnson, N. K., and J. A. Marten. 1992. Macrogeographic patterns of morphometric and genetic variation in the Sage Sparrow complex. Condor 94:1–19.

Klicka, J., and R. M. Zink. 1997. The importance of recent ice ages in speciation: A failed paradigm. Science 277:1666–1669.

Kumar, S., K. Tamura, I. B. Jakobsen, and M. Nei. 2001. MEGA2: Molecular evolutionary genetics analysis software. Bioinformatics 17:1244–1245.

Lambert, D. M., P. A. Ritchie, C. D. Millar, B. Holland, A. J. Drummond, and C. Baroni. 2002. Rates of evolution in ancient DNA from Adélie Penguins. Science 295:2270–2273.

Lovette, I. J. 2004. Mitochondrial dating and mixed support for the "2% rule" in birds. Auk 121:1–6.

Lovette, I. J., E. Bermingham, and R. E. Ricklefs. 2002. Clade-specific morphological diversification and adaptive radiation in Hawaiian songbirds. Proceedings of the Royal Society of London, Series B 269:37–42.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Nei, M., and S. Kumar. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, Oxford, United Kingdom.

Nylander, J. A. A. 2004. MRMODELTEST, version 2. [Online.] Available at www.abc.se/~nylander/mrmodeltest2/mrmodeltest2.html.

Peterson, A. T. 1991. Gene flow in scrub jays: Frequency and direction of movement. Condor 93:926–934.

Peterson, A. T. 1992. Phylogeny and rates of molecular evolution in the *Aphelocoma* jays (Corvidae). Auk 109:133–147.

Peterson, A. T. 1993. Adaptive geographical variation in bill shape of scrub jays (*Aphelocoma coerulescens*). American Naturalist 142:508–527.

Peterson, A. T., and D. B. Burt. 1992. Phylogenetic history of social evolution and habitat use in the *Aphelocoma* jays. Animal Behaviour 44:859–866.

Peterson, A. T., E. Martínez-Meyer, and C. González-Salazar. 2004. Reconstructing the Pleistocene geography of the *Aphelocoma* jays (Corvidae). Diversity and Distributions 10:237–246.

Peterson, A. T., and N. Vargas-Barajas. 1993. Ecological diversity in scrub jays, *Aphelocoma coerulescens*. Pages 309–317 *in* Biological Diversity of Mexico: Origins and Distribution (T. P. Ramamoorthy, R. Bye, A. Lot, and J. Fa, Eds.). Oxford University Press, New York.

Pitelka, F. A. 1951. Speciation and ecologic distribution in American jays of the genus *Aphelocoma*. University of California Publications in Zoology 50:195–464.

Posada, D. 2004. COLLAPSE: Describing haplotypes from sequence alignments, version 1.2. [Online.] Available at darwin.uvigo.es/software/collapse.html.

Posada, D., and K. A. Crandall. 2001. Intraspecific gene genealogies: Trees grafting into networks. Trends in Ecology and Evolution 16:37–45.

Quinn, T. W. 1992. The genetic legacy of Mother Goose—Phylogeographic patterns of Lesser Snow Goose *Chen caerulescens caerulescens* maternal lineages. Molecular Ecology 1:105–117.

Rice, N. H., E. Martínez-Meyer, and A. T. Peterson. 2003. Ecological niche differentiation in the *Aphelocoma* jays: A phylogenetic perspective. Biological Journal of the Linnean Society 80:369–383.

Ronquist, F., and J. P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Rozas, J., and R. Rozas. 1999. DNASP, version 3: An integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15:174–175.

Saunders, M. A., and S. V. Edwards. 2000. Dynamics and phylogenetic implications of mtDNA control region sequences in New World jays (Aves: Corvidae). Journal of Molecular Evolution 51:97–109.

Schneider, S., D. Roessli, and L. Excoffier. 2000. ARLEQUIN: A Software for Population Genetics Data Analysis, version 2.000. Genetics and Biometry Laboratory, Geneva, Switzerland.

Swarth, H. S. 1918. The Pacific Coast jays of the genus *Aphelocoma*. University of California Publications in Zoology 17:405–422.

Swofford, D. L. 1998. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), version 4.0. Sinauer Associates, Sunderland, Massachusetts.

Tajima, F. 1993. Simple method for testing the molecular evolutionary clock hypothesis. Genetics 135:599–607.

Tamura, K., and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10:512–526.

Tarr, C. L. 1995. Primers for amplification and determination of mitochondrial control-region sequences in oscine passerines. Molecular Ecology 4:527–529.

Watson, D. M. 2005. Diagnosable versus distinct: Evaluating species limits in birds. BioScience 55:60–68.

Weir, J. T., and D. Schluter. 2004. Ice sheets promote speciation in boreal birds. Proceedings of the Royal Society of London, Series B 271:1881–1887.

Woolfenden, G. E., and J. W. Fitzpatrick. 1984. The Florida Scrub Jay: Demography of a Cooperative-breeding Bird. Princeton University Press, Princeton, New Jersey.

Zink, R. M., J. Klicka, and B. R. Barber. 2004. The tempo of avian diversification during the Quaternary. Philosophical Transactions of the Royal Society of London, Series B 359:215–220.

Associate Editor: K. T. Scribner